08-29-05

IFW

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Sheldon R. Pinnell

U.S. Application No.: 10/691,840

Filed: 10/23/2003 Attorney Ref.: SKIC02

Title: "SKIN-CARE COMPOSITION"

Express Mail mailing label no. ER 893388675 US
Date of Deposit August 26, 2005
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Lynn E. Barber
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RESPONSE TO OFFICE COMMUNICATION UNDER 37 CFR 1.136(a)

Mail Stop Sequence
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office communication dated July 27, 2005 in the above-referenced pending patent application, Applicant hereby submits, under 37 CFR 1.1136(a), a copy of the Notice to Comply, a separate paper Sequence Listing, and a copy of the Sequence Listing in computer readable form. An amendment directing entry of these into the specification, and amending the language of the specification is also enclosed.

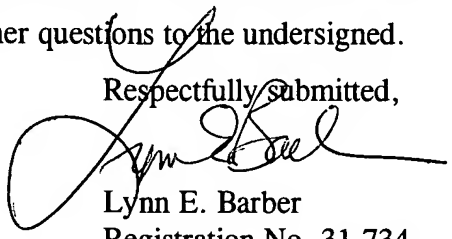
Applicant has also enclosed a copy of the product literature where information on the sequence is found (reference given in Sequence Listing).

Further, the undersigned attorney-of-record for Applicant hereby states, under 37 CFR

1.821, that the content of the paper and computer readable copies are the same, and include no new matter.

It is submitted that this submission is fully compliant with the Office communication requirements. Please direct any further questions to the undersigned.

Respectfully submitted,



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Notice to Comply	Application No.	Applicant(s)	
	10/691,840	PINNELL, SHELDON R.	
	Examiner	Art Unit	
	Patricia Leith	1655	

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Applicant must file the items indicated below within the time period set the Office action to which the Notice is attached to avoid abandonment under 35 U.S.C. § 133 (extensions of time may be obtained under the provisions of 37 CFR 1.136(a)).

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

☒ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998).

☒ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).

☒ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable from of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).

☐ 7. Other:

Applicant Must Provide:

☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".

☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.

☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216
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sederma

SEDERMA PATENT FR 0201967 1/2

Combating bags under the eyes

EYELISS™

SEDERMA - 29, rue du Chemin Vert - 78612 LE PERRAY-EN-YVELINES
Tel.: 01.34.84.10.10 - Fax: 01.34.84.11.30

CONTENTS

04/2002/V1

EYELISS™

SYNOPSIS

Description: Combination of 3 complementary active substances in solution.

INCI name: Water (*Aqua*) (and) Glycerin (and) Hesperidin Methyl Chalcone (and) Steareth-20 (and) Dipeptide-2 (and) Palmitoyl Tetrapeptide-3

Objectively demonstrated activity:

- Published data on hesperidin methyl chalcone
  - *Ex vivo* study (Tarayre and Lauressergues, 1976): very significant 25% decrease in capillary permeability.
  - *In vivo* study (Von Rudovsky, 1989) : strengthening of the capillary wall by decreasing the filtration leading to edema.

- *In vitro* studies
  - Inhibition of angiotensin-converting enzyme (ACE) by dipeptide Val-Trp: 85% inhibition of ACE with 0.001% dipeptide VW equivalent to use of 1% **EYELISS™**.
  - Anti-inflammatory activity of peptide Pal-GQPR:
    - Regulation of basal levels of IL6 produced by keratinocytes.

- *In vivo* studies

  Clinical study in 20 volunteers (gel containing 3% **EYELISS™**, twice daily application for 56 days).
  - 3D morphometric measurements by fringe projection and signal acquisition using a high-resolution CCD camera (EoTech, France).
  - Self-assessment questionnaire completed by the panelists.
  - Illustrative digital imaging.

Recommended dose for use : 3%

Safety (reports on request)

HET CAM test
Patch test on humans
RIPT
AMES test
Neutral Red Cytotoxicity

1. INTRODUCTION

BAGS UNDER THE EYES: AN EXCESSIVELY VISIBLE SIGN OF AGING

Called into action ten thousand times a day to moisten the eyes, prey to emotions that contract (worry, stress) or stretch them (surprise, joy), subjected to the blinking reflexes on variation in light intensity, the eyelids are indubitably, together with the lips, one of the two most mobile cutaneous surfaces of the body.

It is thus not at all surprising that the very first signs of aging, the first circulatory disorders and the greatest sensitivity to irritation affect, as a priority, those cutaneous membranes whose thickness is only equivalent to five sheets of paper (0.5 mm on average and 6 to 7 cell layers).

Sagging of the skin, with the formation of bags, rings and wrinkles under the eyes, affects men and women equally ...

Transient disorders in an excessively frenetic lifestyle, rings under swelling of the lower eyelids are relatively well tolerated in young individuals. However, those signs become lasting as time passes and become unsightly and poorly accepted in adults. Cosmetic intervention is then sought to restore a bright and fresh look.

Although the appearance of the contours of the eyes may be enhanced by products with a 'flash effect' that immediately smooth the skin of the bags, it is more important to be able to offer consumers a care product which treats that particularly fragile zone of the face and restores its tone and youthful appearance.

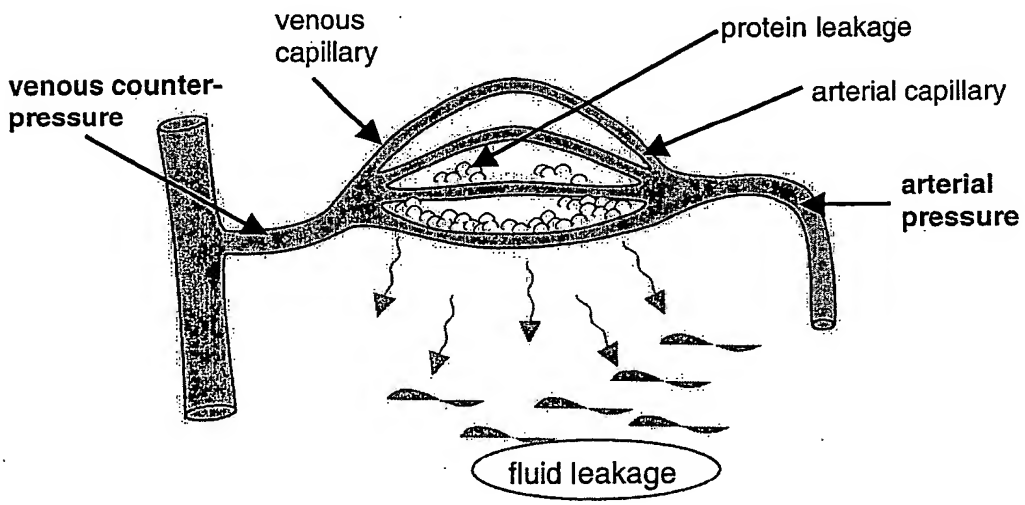
THE CAUSES AND SYMPTOMS OF BAGS UNDER THE EYES

Several factors contribute to the emergence of bags under the eyes and their maintenance and accentuation over time:

1. Capillary fragility

The eyelid is crossed by a fine, dense network of arterial and venous capillaries. Following successive irritations and transient disequilibria such as tiredness, hypertension or the intake of certain drugs, together with the effects of age, the vessel walls become fragile and plasma fluid leaks from the vascular bed resulting in interstitial fluid accumulation: edema or bags under the eyes come into being.

Edema formation



The fragility of the capillary loops results in increased permeability of the walls, which allow excessive leakage of water and proteins.

The diagram above shows that decreasing capillary permeability to prevent extravasation is a key factor in countering the emergence of suborbital bags.

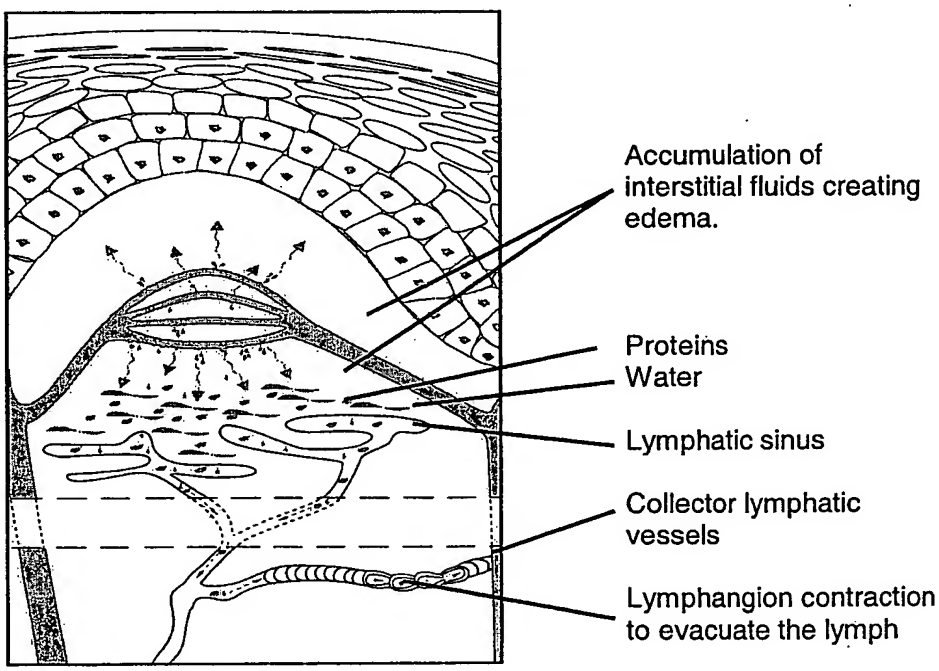
2. Lymphatic drainage

The accumulation of interstitial fluids, which are not eliminated by the lymphatic system, results in local overload and distension of the skin : a bag is formed, swollen with water, protein and various ions.

The lymphatic drainage of the eyelids, like that of the other tissues, is ensured by a specific network of small, superficial sinuses close to the cutaneous capillaries which merge to form lymph canals, then lymph vessels in the inferior hypodermis.

Mechanically pulsed by neighboring muscular fibers towards the superficial sinuses, the interstitial fluid flows are subsequently collected by the lymphatic network and transported by the pulsatile action of lymphangions (*vasa lymphatica*), a sort of specific contraction module in the lymph canals (the lymph flow rate is 6 mm/s).

Lymphatic drainage



This particular system is responsible for lymphatic drainage in the body as a whole, with daily recycling of 20 to 50% of blood plasma and a flow rate of about 2 L/24 h.
Defective drainage greatly contributes to the formation of bags under the eyes.

3. Aging : inflammation and loss of cutaneous tone

As described above, the constant use of the eyelids and the fineness of their epithelium fairly rapidly result in tissue sagging with weakening of the supporting structure around the microvessels and increases in dilated areas that retain edema.

An inflammatory component generally accompanies infiltration of the tissues and acts as an exacerbating factor for edema. This component must not been neglected.
We are aware that aging is generally accompanied by an increase in certain pro-inflammatory cytokines such as IL6 due to decreased control exerted by hormone DHEA. Cytokines are also activated daily by UV radiation and the pro-inflammatory effect is additional to that of aging.

The combination of those three factors is above all responsible for the phenomenon 'bags and rings under the eyes'.
In order to combat it, we have chosen an overall strategy, a holistic approach which combines several active substances intended to manage each of the major etiologies described above.

SEDERMA has thus developed **EYELISS™**, an 'anti-bag' active substance, to treat sagging and swollen eyelids and thus enhance the smoothness (wrinkles), firmness and tone of the skin.

2. DESCRIPTION OF EYELISS™

A MULTI-TARGETED APPROACH FOR OPTIMUM EFFICACY

To combat the emergence of bags under the eyes and decrease their volume once they have become established, it is necessary to act on the physiological deficiencies giving rise to the phenomenon:

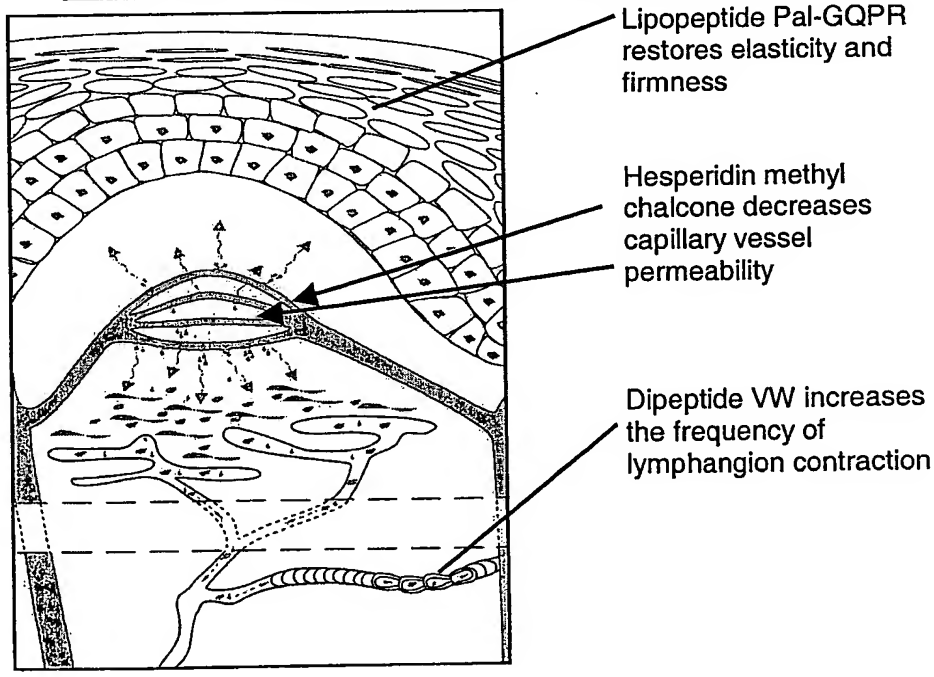
- capillary permeability, which promotes water and protein leakage into surrounding tissues: formation of edema
- tissue sagging and inflammation contributing to fluid stasis
- and, lastly, insufficiency of the lymphatic drainage, which no longer effectively eliminates the fluids accumulated.

EYELISS™ is supplied in the form of a light orange, clear, aqueous solution, which contains three active substances:

- hesperidin methyl chalcone (5%),
- dipeptide VW (0.1%)
- Pal-GQPR (300 ppm)

The product, **EYELISS™**, is a combination of the three specific and innovative active substances, whose actions are described below. **EYELISS™** is designed for the preventive and reparatory treatment of bags under the eyes.

Action sites of the active substances in EYELISS™

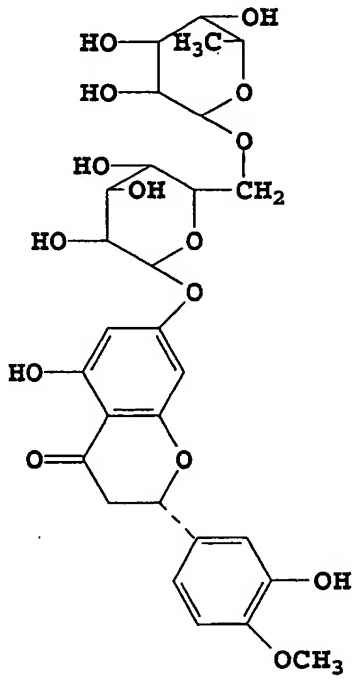


2.1. Hesperidin methyl chalcone

Numerous products of natural origin (flavonoids, *Ruscus* extract, horse chestnut extract, etc.), sometimes chemically modified (e.g. hydroxyethylrutoside), are used to strengthen distended and permeable vascular walls.

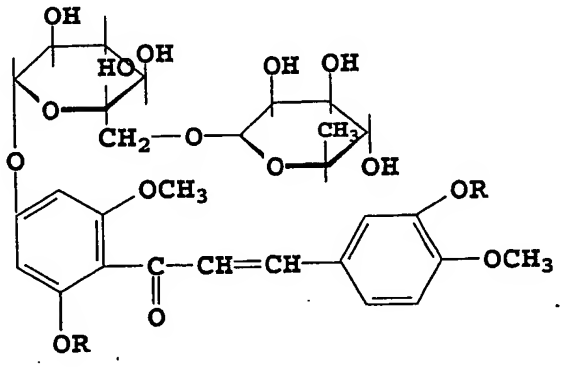
Hesperidin and its derivatives are flavonoids present in citrus fruits - lemons, oranges - where they may account for up to 14% of the fresh weight (BARTHER, 1988).

Hesperidin



Hesperidin methyl chalcone, a methylated form of hesperidin, selected to combat capillary extravasation, has the following chemical formula:

Hesperidin methyl chalcone



The anti-edematous proprieties of hesperidin methyl chalcone have been reported in numerous publications (GALATI, 1994; GARG *et al.*, 2001) and are exploited in various vasculoprotective formulations.

It has also been shown that hesperidin methyl chalcone decreases transcapillary diffusion of a model molecule such as dextran in the hamster (BOUSKELA, 1993) and reduces capillary permeability in man (RUDOVSKI, 1989).

Hesperidin methyl chalcone is therefore present at a high concentration in **EYELISS™** due to its established venotonic properties.

2.2. Dipeptide VW

As indicated in the introduction, insufficient lymphatic drainage strongly contributes to the formation of bags under the eyes. We therefore investigated how to correct that insufficiency.

The contraction of lymphangions, necessary to ensure drainage, is dependent on an endogenous nonapeptide : bradykinin which activates the ß2-receptors of the lymphangion and increases the frequency of contractions (DOBBINS, 1990, and YOKOYAMA, 1996).

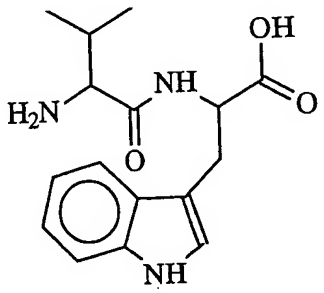
Bradykinin is naturally produced by the body and the system is thus normally activated. However, the life of that peptide is relatively short because it is dependent on the main degradation enzyme, angiotensin converting enzyme or ACE (WARREN, 1995): ACE, through its action, thus controls the frequency and intensity of the pulsations.

It may reasonably be considered that deficient lymphatic circulation results from slowed drainage: the contractions of the lymphangions must therefore be re-triggered or prolonged in order to enhance the efficacy of interstitial water pumping.
One relatively simple method is to decrease the activity of ACE so as to locally prolong the lifetime of the effector nanopeptide and thus strengthen the contractions or prolong their duration.

The enzymatic activity of ACE may be inhibited by the short peptide sequences described by SAITO[1] (1994). Those peptides have been isolated from *sake*, the traditional Japanese fermented rice beverage whose nutritional and antihypertensive properties have been demonstrated experimentally.

Among those peptides, dipeptide valyltryptophan (VW) is particularly effective in the hypertensive rat presenting excessive ACE activity: the performances obtained are similar to those of a reference product, Captopril, (SAITO[2], 1994).

We therefore selected the dipeptide, Val-Trp, also known as VW, whose structure is as follows:

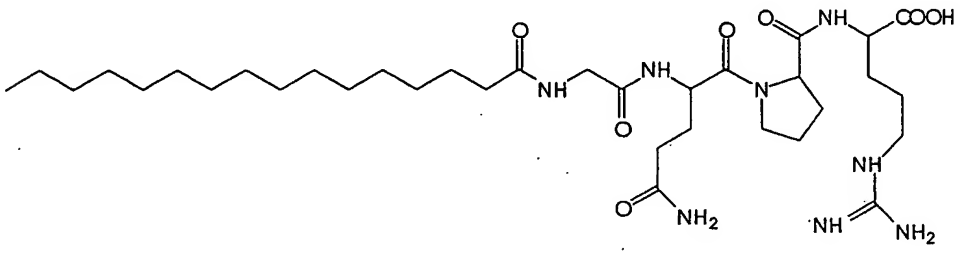


to stabilize the endogenous mediator of lymphangion contraction by inactivating ACE, which degrades it.

ACE inhibition has a second complementary effect: as the name, angiotensin converting enzyme indicates, ACE converts angiotensin I, an inactive precursor, to a very potent peptide, angiotensin II. Angiotensin II mainly acts as a vasoconstrictor to increase blood pressure. Angiontensin II also increases fluids retention in the body, thus interfering with tissue drainage. Thus, by inhibiting ACE, we also act on water retention by enhancing evacuation.

2.3. Peptide Pal-GQPR

In order to reinforce the fragile cutaneous tissue under the eyes, a third active substance has been included in **EYELISS™**, peptide Pal-GQPR (Palmityoyl-Gly-Gln-Pro-Arg). Pal-GQPR is a fragment of a human immunoglobulin which specifically acts on secretion of cytokine IL6.



The peptide, the active substance in RIGIN™ (cf. corresponding SEDERMA technical dossier), induces a reduction in the basal or UV radiation-induced secretion of IL6, but also has a demonstrated cutaneous restructuring effect *in vivo*.

Pal-GQPR was tested on a panel of 16 volunteers who used the peptide daily. The results showed:

- a 17-19% increase in firmness
- a 27-39% increase in elasticity
- a decrease in wrinkles : -56% at 75 μm

A study using the DNA-array method showed that the peptide activates certain genes involved in tissue cicatrization, in particular cicatrization of the skin (SEDERMA/Biopredic).

Those very interesting properties are perfectly pertinent to the problem of bags under the eyes. We therefore incorporated the lipopeptide in **EYELISS™** in order to combat the influence of inflammation in the bag symptom and restructure the tissues by activating the cicatrization process.

It is sometimes stated that swelling of the lower eyelids may also be due to an accumulation of fat in the lower peri-orbital area.

We must therefore distinguish between edema and fat accumulation. The former is enhanced by the nighttime horizontal position, while the latter remains constant throughout the day. The combination of **EYELISS™**, whose properties demonstrated *in vivo* are addressed in the following section, may therefore be combined with LIPOCARE™, PLEURIMINCYL™, CoAXEL or any other SEDERMA lipolytic active product, when a more aggressive (esthetic surgery) approach is not required.

3. EFFICACY TESTS

3.1. Published data on hesperidin methyl chalcone

Out of the numerous studies conducted on hesperidin methyl chalcone, only the characteristic data of 2 studies : *ex vivo* on fragile skin biopsy material, and an *in vivo* study in man, will be considered hereafter.

3.1.1. *Ex-vivo* study (Tarayre and Lauressergues, 1976)

This study was conducted on excised skin. The study consisted in experimentally inducing capillary fragility, then comparing the increase in capillary permeability in rats treated and not treated with hesperidin methyl chalcone.

Protocol

The increase in capillary permeability was induced by intradermal injection of histamine.

The irritant histamine injections were conducted locally to induce wheals. The rats had previously been administered an extravasation marker, Evans blue, which binds to albumin, by the systemic route.

After 30 minutes, the skin of the animals was excised and placed, in vitro, in the presence of formamide and incubated at 65°C for 24 hours.

Evans blue release was monitored using a spectrophotometer adjusted to 620 nm.

Results

The increase in capillary permeability in the control cases and the cases treated orally with hesperidin methyl chalcone (HMC 40 mg) were compared.

	Capillary permeability	% change
Control	0.411 ± 0,01	
HMC-treated	0.307 ± 0,01	- 25%
Significance	p<0.01	

A very significant 25% decrease in capillary permeability was obtained in the presence of hesperidin methyl chalcone.

Histamine induces the formation of pores between endothelial cells. The above data clearly show the protective effect of HMC *vis-à-vis* capillary fragility.

3.1.2. *In vivo* study (Von Rudovsky, 1989)

A placebo-controlled study was conducted in 20 volunteers to demonstrate the vasculoprotective effect of a combination of *Ruscus* extract and hesperidin methyl chalcone (*Ruscus*-HMC). The parameters monitored were the decrease in venous reflux forming stasis and the strengthening of the fragile vessel walls, by measuring the increase in hydrostatic pressure.

The improvement in venous return and the decrease in capillary fragility were studied following administration of the 2 compounds by the oral route alone (150 mg) and in combination (300 mg).

Protocol

Venous return was investigated using a hemodynamic signal, PPG, which enables measurement of passive blood filling during the variations in venule reflux associated with venous insufficiency.

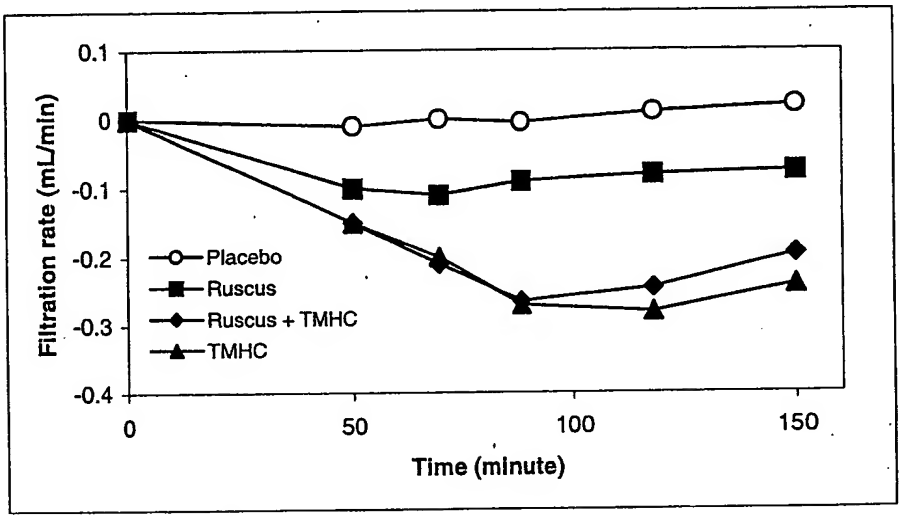
Capillary permeability was monitored by plesthysmography, a method in which the increase in skin volume, related to water and protein leakage from capillaries, is measured.

Results

Ruscus and HMC induced different results.

HMC decreased capillary leakage by 0.3 mL/min over the observation period. The decrease was highly significant.

Combination with *Ruscus* extract did not improve the results. (cf. fig. below).



In the *in vivo* test, a clear differentiation between the effect of *Ruscus* and that of HMC on capillary permeability was observed. HMC was 3-fold more potent than *Ruscus* with respect to slowing water and protein leakage (edema formation rate).

These results (cf. von Rudovsky, 1989, for further details) clearly show the value of HMC treatment for venous insufficiency. HMC may be combined with *Ruscus* extract (for the latter's proven venotonic effect). The strengthening action on capillary walls decreases the filtration resulting in edema.

3.2. *In vitro* studies

3. 2. 1. ACE inhibition by dipeptide Val-Trp

Principle

Angiotensin converting enzyme (ACE) catalyzes the formation of angiotensin II (8 amino acids) from angiotensin I (10 amino acids) and the degradation of bradykinin (9 amino acids) to BK1-7 (inactive metabolite consisting in 7 amino acids).

A synthetic analog of the natural substrates was used: tripeptide FAPG (furyl-acryloyl-phenyl-ala-gly-gly) which is converted to FAP (loss of 2 glycines) and whose emergence was monitored by spectrophotometry (λ = 340 nm). The enzymatic activity was directly proportional to the quantity of FAP formed.

Protocol

The ACE enzymatic activity assay kit method (Sigma) consists in incubating a synthetic substrate (FAPG) in the absence (control) and in the presence of dipeptide VW.

Incubation was conducted at 37°C and FAP formation was measured at the endpoint, T = 5 minutes, for an enzyme concentration of 10^{-2} units/mL.

Results

The variation in absorbance in the presence of dipeptide VW, at various concentrations was compared to the variation for the dipeptide-free control. The values are expressed in percent of the control activity.

The results are the mean values for n = 3 determinations.

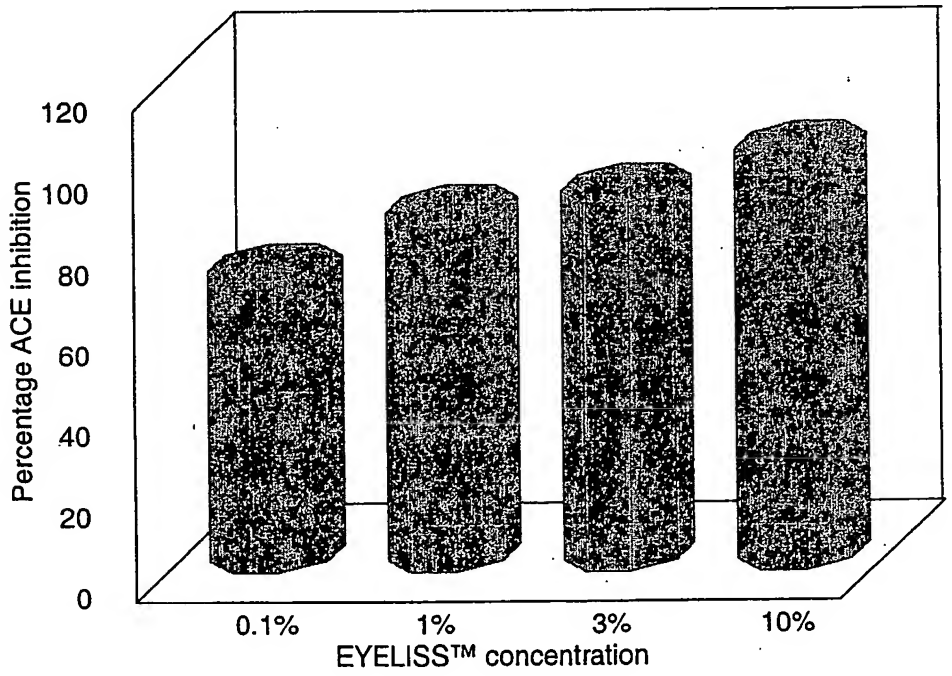
EYELISS™ equivalence	Dipeptide VW concentration	ACE inhibition
Eyeliss™ 0,1%	0.0001%	-71%
Eyeliss™ 1%	0.001%	-85%
Eyeliss™ 10%	0.01%	-100%

As expected, dipeptide VW was clearly shown to be an ACE inhibitor.

The inhibition was concentration-dependent with marked inhibition even for low dipeptide concentrations.

Inhibition was total with 0.01% peptide VW and very marked, 85%, with 0.001% dipeptide VW.

By interpolation, the ACE inhibition with 0.003% dipeptide VW, equivalent to use of 3% **EYELISS™**, was calculated to be 90%.



3. 2. 2. Anti-inflammatory activity of peptide Pal-GQPR

The importance of cytokine balance in the control of the processes of immune and inflammatory reactions has now been clearly established.
Interleukin 6, in particular, plays a key role for the inflammatory reactions induced by UV radiation or related to age (2-fold increase in circulating levels), locally creating chronic pro-inflammatory conditions.

It has been shown that epidermal cells synthesize that cytokine and that synthesis may be regulated, among other mediators, by DHEA, a hormone of youth.

In that context, peptide Pal-GQPR was developed since it exerts a specific action on basal or UV-induced IL6 as was shown in the following tests:

a) Regulation of basal IL6 levels for keratinocytes

Protocol
Human keratinocytes were cultured up to sub-confluence, then exposed to Pal-GQPR for 48 hours. The IL6 levels were determined using an ELISA method.

Results
The results obtained in the absence (control) or presence of Pal-GQPR were analyzed and the percentage inhibition calculated. The mean values were determined for a series of 3 assays.

Variation of basal IL6 level in the presence of peptide Pal-GQPR

Pal-GQPR (ppm)	Decrease in IL6 (% basal level)
10	-15%
15	- 20%
30	- 25%

A marked decrease in basal IL6 level was observed as of a concentration of 10 ppm. The reduction reached 25% when the peptide was present at a concentration of 30 ppm.

b) Regulation of UV radiation-induced IL6 levels

Protocol

After 24 hours of culture in the presence or absence of Pal-GQPR, the cells were subjected to UVB radiation (35 mJ/cm^2), then cultured with fresh medium for a further 24 hours.

In the absence of Pal-GQPR, the IL6 levels were increased 20-fold. In the presence of the peptide, IL6 levels were reduced to lower values:

Control of excess IL6 production in the presence of Pal-GQPR

Mean values for n = 2 assays

Pal-GQPR (ppm)	Decrease in induced IL6 level
10	-33%
15	-37%
30	-60%

The results of the test show that a reduction, by one third, in the overproduction of IL6 by UV was obtained for a peptide concentration of 10 ppm. A 2/3 reduction was obtained when the peptide concentration was 30 ppm.

Given the above results, Pal-GQPR was included in the product, **EYELISS™**, to combat the inflammation related to peri-orbital edema, thus antagonizing the age-related increase in IL6 (elevation of the basal level) and reducing the overproduction on daily exposure to UV radiation.

equivalence	Pal-GQPR (ppm)	Anti-inflammatory activity
3% EYELISS™	10ppm	-15% basal IL6
3% EYELISS™	10ppm	-33% UV radiation-induced IL6

3. 3. *In vivo* study

Principle

A really effective care product for bags under the eyes must have the capability of treating subjects suffering from the chronic (and not occasional) bags under the eyes that have formed over time, with an efficacy that is enhanced over time: that objective was pursued with **EYELISS™**.

Efficacy assessment methods

- 3D morphometric measurements by fringe projection and signal acquisition using a high-resolution CCD camera (EoTech, France).
- Self-assessment questionnaire completed by the panelists.
- Illustrative digital imaging.

Protocol

The clinical trial was conducted in 20 female volunteer subjects of mean age **51 ± 6 years presenting with chronic bags under the eyes.**

The inclusion criteria were: healthy skin in the determination zone, prohibition on the use of aspirin or any other anti-inflammatory or anti-histamine treatment of systemic corticosteroids; no use of decongestant products at peri-ocular level, no change in cosmetic habits for both hygiene and care products.

The study had an open-label design and each subject acted as her own control. The results obtained at baseline (T0) were compared to those obtained at time points T28 and T56 days.

The volunteers applied **EYELISS™** as a 3% formulation in gel (appendix 1) morning and evening for 2 months. The product was applied by dabbing with the fingers, under each eye, from the lateral extremity to the medial extremity.

The study flow chart was as follows:

T0	T 28 days	T 56 days →
3D measurements Photographs	3D measurements Photographs	3D measurements Photographs Self-assessment questionnaire

Note:

The determinations on T28 and T56 were conducted in the absence of the product, the last application having taken place the previous day.

The measurements conducted were thus:

- free from any interference related to the cosmetic application technique (drainage due to a mechanical effect)
- taking into account the peri-orbital stasis that is known to be amplified after a night in the prone position.

In that manner, the measurement made reflects a real decrease in the bags under the eyes and the effect is directly related to the product.

Results

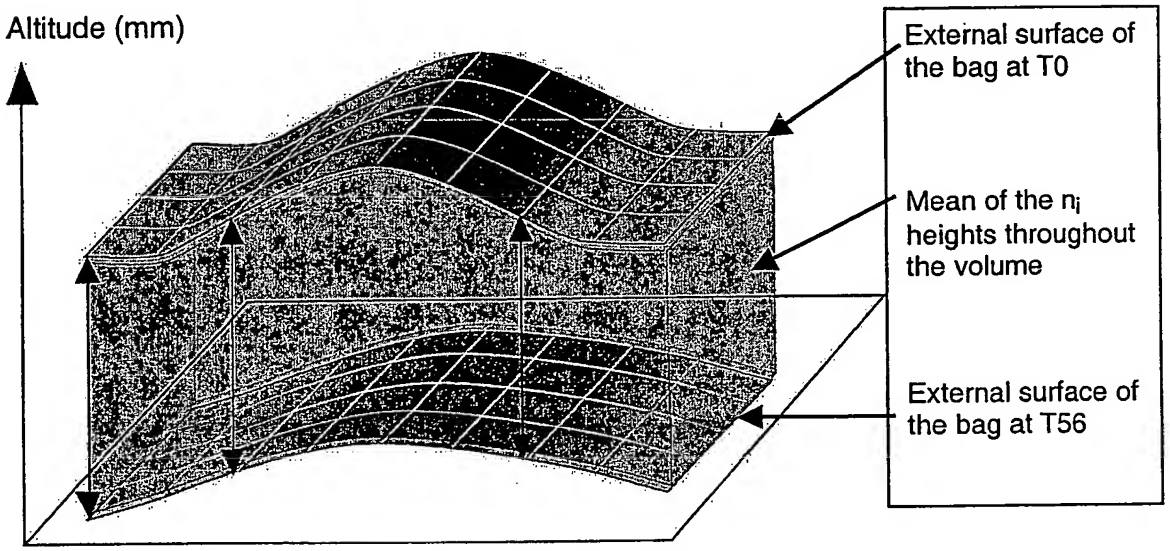
1. Fringe projection measurements

Safety

The product was perfectly tolerable for all the subjects.

3D morphometric measurements

The diagram below illustrates the nature of the measurements made:



The parameter generated by the analysis software package (Innov Metric Software INC, USA) from the 3D-acquisition data consists in the mean distance separating the 2 surfaces of the bags pre- and post-treatment. On the diagram, the parameter is, in fact, the mean of the n distances separating the initial surface (red) from that at T28 or T56 (green).
For each subject, a mean value representing the lowering of the peri-orbital arch (wall of the bag) was thus generated.
An overall mean for n = 20 subjects was then calculated for time points D28 and D56.
The significance of the results was evaluated using Student's t test for paired series at a significance level of 5%.

Mean lowering (n = 20) of the peri-orbital arch
after 1 and 2 months of treatment with **EYELISS™** 3% gel

	Lowering of the bag vs. T0 (mm)	
	T28 – T0	T56 – T0
Mean altitude	-0.08	-0.20
Subjects presenting with an improvement (%)	65%	70%
Significance T0	p<0.05	p<0.01
Significance T56/T28	p<0.05	

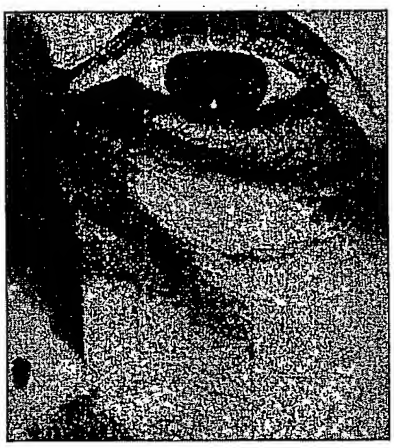
The results clearly show a highly significant decrease in bag volume. The decrease was observed for over 65% of the subjects after 28 and 56 days of treatment.

After 56 days, 14 subjects, i.e. 70% of the panel, showed reduction in the bags under the eyes, with, for 2 volunteers, decreases of -0.63 and -0.70 mm.
For 4 subjects, the reductions exceeded -0.42 mm.

To illustrate the results, a few digital photographs are included below:

T0

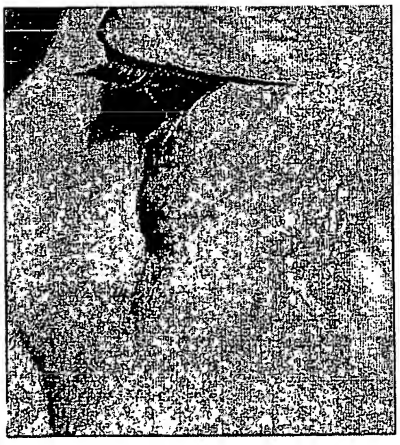
T56



T0

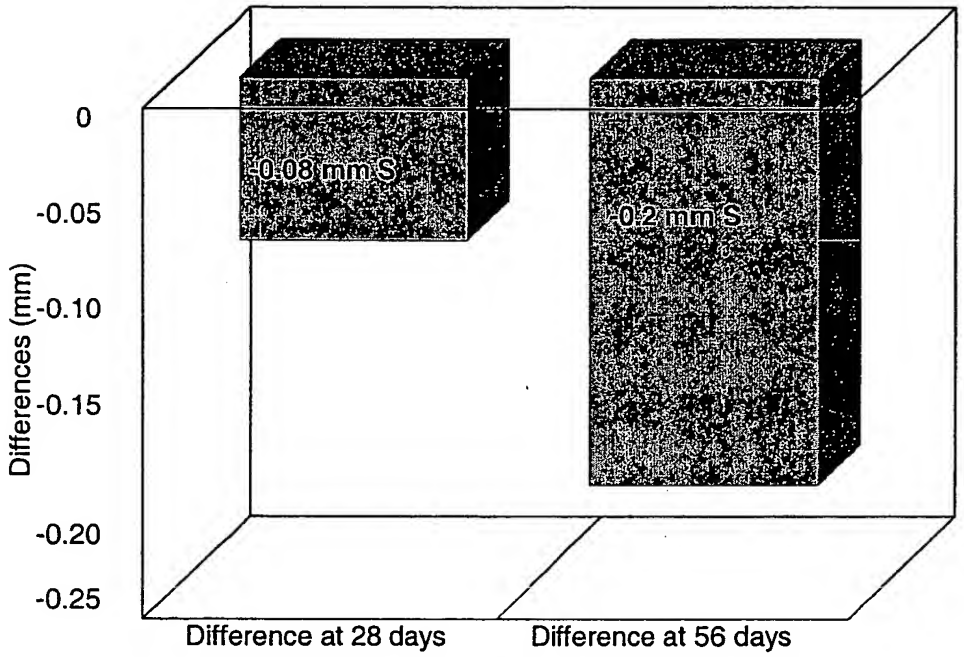
T56

T0



T56

The bar chart below summarizes the results obtained:



S: significant difference vs. baseline (T0) at the 5% level

Conclusion

The 3D morphometric analysis by fringe projection evidenced real efficacy of the **EYELISS™** product on reduction in the volume of chronic bags under the eyes in subjects of mean age 51 years after 56 days.
The very marked time course of the regression between days 28 and 56 suggests that the performance will be even more remarkable over longer treatment durations.

2. Self-assessment by the subjects

In a study like the present one, it is very important to correlate the instrumental determinations of the reduction in the bags under the eyes with the volunteers' assessment of product efficacy.

Two aspects were investigated by the questionnaire: first, the hedonistic aspect of the product (cosmetic acceptability), and, secondly, the satisfaction relative to what was expected of the product.
The questionnaire was completed at the end of the study (D56).

For each of the questions raised, the volunteers expressed their agreement in the following manner:

A not at all in agreement
B not really in agreement
C in agreement
D totally in agreement

The frequency of the various response modalities was calculated. Then, for each item, the sum of the frequencies A+B and C+D were compared, using the Chi^2 test, and the significance of the difference determined at the 5% level.

Results

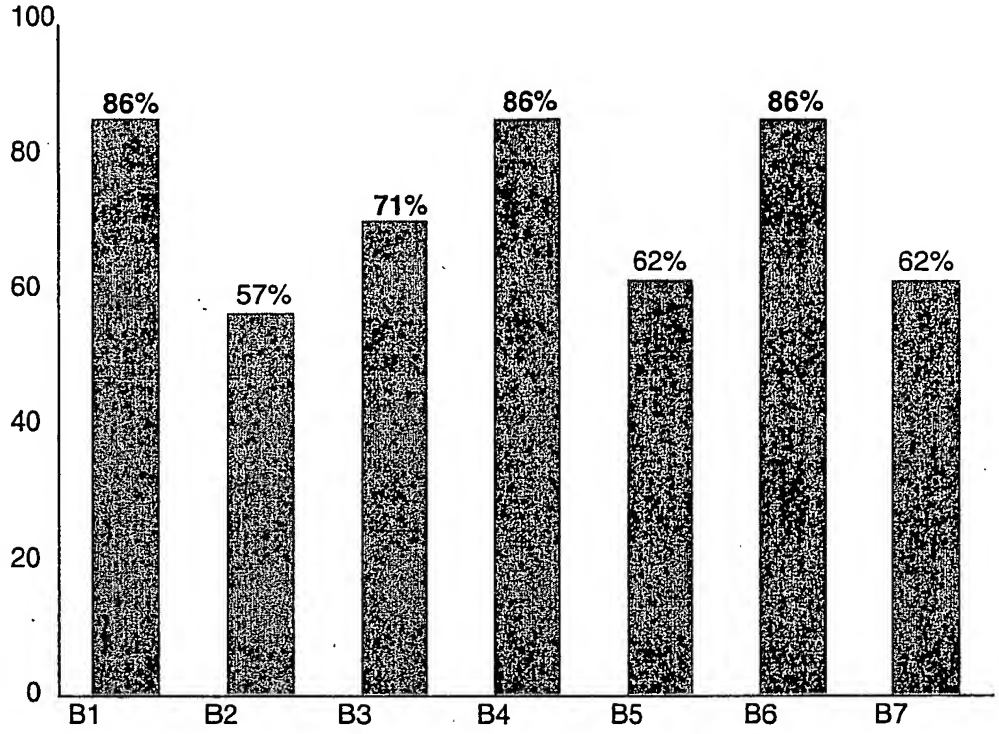
Hedonistic aspect:
Out of the subjects questioned, 86% considered that:

- the product did not stick to the skin after absorption
- the product procured a feeling of freshness
- the product was easy to apply

the assessments were significant ($p < 0.05$)

- 62% of the panel appreciated the rapid penetration of the product and the silky appearance of the skin.
- The product texture was rated pleasant by 71% of the volunteers ($p < 0.05$).

The set of assessments is shown in the plot below:



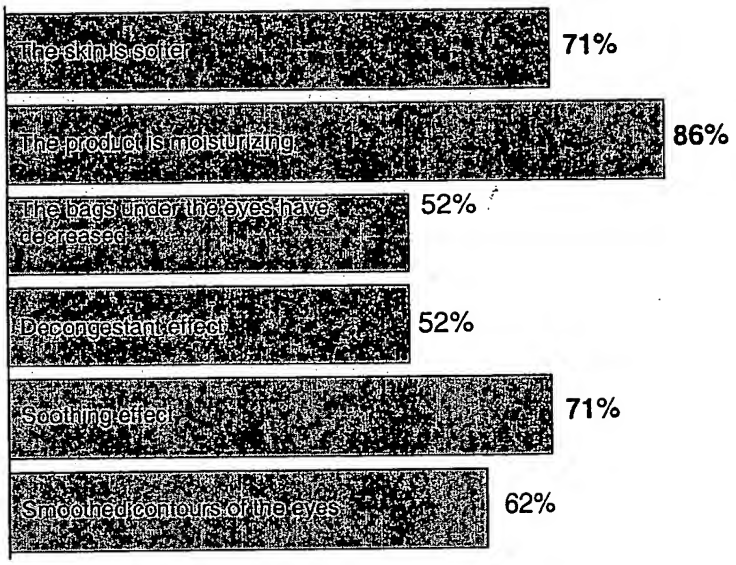
B1 After production absorption, the skin is not sticky
B2 The product has a pleasant fragrance
B3 The product has a pleasant texture
B4 The product procures a feeling of freshness
B5 The product penetrates rapidly
B6 The product is easy to apply
B7 The product procures a silky appearance of the skin

Product efficacy

The subjects questioned formulated the following remarks:
The contours of the eyes were considered smoothed by 62% of the volunteers with a reduction in the bags under the eyes (52%) and a decongestant effect (52%).

- the product moisturizes the skin : 86% satisfaction
- the skin is softer : 71% satisfaction
- the product has a soothing effect : 71%
  significant responses ($p < 0.05$).

After 56 days of application, the anti-bag product was rated effective by 52% of subjects.



In short, for the 2 sections of the questionnaire, irrespective of the question put, most of the responses were positive.
The product has good cosmetic acceptability and is recognized as effective in the treatment of bags under the eyes.

Overall, the results show the following strengths for EYELISS™:

The product is moisturizing, procures softness and is soothing.
The majority of the volunteers considered the product effective. It decreased the size of the bags under the eyes, had a decongestant effect and smoothed the contours of the eyes.

4. CONCLUSION

To maintain or restore the freshness and youthfulness of facial expression, the contours of the eyes must be tonic, free from sagging, smooth, and free from bags under the eyes.

Eye contour, while it may betray age or excessively frenetic life style, is a very fragile zone whose appearance reflects all the minor, local, venous, lymphatic and cutaneous disequilibria.

The cosmetic efficacy of a product to alleviate bags under the eyes must address the main components associated with the emergence and maintenance of that unsightly phenomenon, which is observed in both men and women:

- capillary fragility which allows the leakage of plasma fluid and the formation of edema
- cutaneous sagging promoting interstitial stasis of fluids and the inflammatory condition that is associated
- local insufficiency of lymphatic drainage, which no longer eliminates the fluids accumulated.

To overcome each of the above deficiencies, it was necessary to combine appropriate specific compounds in a single product. The active substances selected were:

- hesperidin methyl chalcone, which decreases fluids leakage by strengthening the capillary microvessel barrier
- dipeptide VW which mobilizes fluid in the tissues and drains it by activating the lymphatic pump
- peptide palmitoyl-GQPR, which reduces local inflammation and restores cutaneous firmness and elasticity.

The soundness of the concept was demonstrated by a clinical study in 20 volunteers of mean age 51 years presenting with chronic bags under the eyes.

The 3D morphometric determinations (fringe projection) conducted the day after application enabled elimination of the immediate reduction associated with the cosmetic intervention and thus took into account the peri-orbital stasis amplified by a night in a prone position.

After 56 days of twice-daily treatment with EYELISS™, 3% formulation, a significant reduction in bag volume was obtained in 70% of the volunteers.
Overall, the mean reduction obtained was -0.2 mm, with, for 4 subjects, a differential of -0.4 mm and, for 2 subjects, -0.6 and -0.7 mm.

The self-assessment questionnaire and the volunteers' spontaneous reports support the results with 'smoothed contours of the eyes' (62%), reduction in the bags (52%) and a decongestant effect reported by 52% of the volunteers.

To slow bag formation or reduce chronically-formed bags, we recommend use of EYELISS™ at a concentration of 3%.

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APPENDIX 1

Formulations used for the clinical trial:

Starting materials	INCI name	Suppliers	w/w
Phase 1			
Demineralized water	Water (*Aqua*)		qsp 100
Carbopol Ultrez 10	Carbomer	BF Goodrich	0.20
Phase 2			
Glycerin	Glycerin		5.00
Preservatives			qs
Phase 3			
Natrosol 250M	Hydroxypropylcellulose	Aqualon	0.20
Phase 4			
Crodamol CAP	Ceteraryl Octanoate	Croda	6.00
Pemulen TR2	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	BF Goodrich	0.20
Phase 5			
Potassium sorbate	Potassium Sorbate		0.10
Phase 6			
Demineralized water	Water (*Aqua*)		4.00
10N NaOH			0.46
Phase 7			
Crillet 1	Polysorbate 20	Croda	0.50
Fragrance			qs
Phase 8			
EYELISS™	cf. synopsis	SEDERMA	3.00